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Dynamic properties of bacterial flagellar motors

TETHERED bacteria[1] rotate at the angular velocity at which the torque generated by the flagellar motor[2] is balanced by the torque due to the viscous drag. In general, $M = b\eta\Omega$, where M is the torque, η is the viscosity, Ω is the angular velocity, and b is a coefficient which depends on the size and the shape of the cell, the position of the axis of rotation, and the distance between the cell and the wall. For a sphere of radius a (not too close to the wall) $M = 8\pi\eta a^3\Omega$ (ref. 3). Viscous forces are so large in comparison with inertial forces[4] that Ω will change with M virtually instantaneously; any discontinuities in the one will be evident in the other. Consider a cell of radius a and uniform density ρ rotating at an angular velocity Ω_0; if its motor is suddenly disengaged, Ω will decay exponentially to 0 with a time constant $\rho a^2/15\eta$, and the cell will stop in $\Omega_0\rho a^2/15\eta$ radians. For *Escherichia coli* this is less than a millionth of a revolution. The cell also is subject to rotational diffusion, but this will be evident only if the coupling between the flagellum and the body of the cell is fluid. The root-mean-square deviation in the angular position is $(2Dt)^{1/2}$, where D is the rotational diffusion constant and t is the time. For a cell which can rotate freely, $D = kT/b\eta$, where k is Boltzmann's constant and T is the absolute temperature.

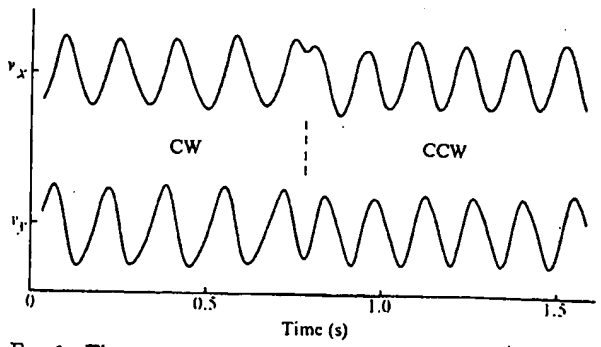

FIG. 1 The rotation of a polyhook mutant bound to the top window of the tracking chamber. The cell rotated clockwise (CW) at 6.2 r.p.s. and then counterclockwise (CCW) at 7.2 r.p.s. (The x velocity lags the y velocity for CW rotation but leads it for CCW rotation; the phase shift is 90°.) *E. coli* strain MS1381 was grown on minimal medium containing glycerol as described by Silverman and Simon[6], washed twice with a solution containing 10^{-2} M potassium phosphate (pH 7.0) and 10^{-4} M ethylenediamine tetraacetate, and suspended in a similar medium containing 0.067 M sodium chloride (10^8 cells ml^{-1}). Antipolyhook antibody was added to a dilution of 1:20,000, and the window was immersed in this mixture. It was removed 20 min later and inverted onto the top of a tracking chamber (filled with the suspension medium). The cells were tracked at 32°C. The microscope readout voltages (ref. 5, Figs 1 and 5) were amplified, differentiated, and displayed on a strip-chart recorder. The frequency response of this system was checked with sine waves of amplitude 0.05 and frequency 20 times that of the signals which generated the recording shown in the figure; the test signals were readily detectable. Note that the signals generated by the rotation of the cell are completely smooth.

The rotation of *E. coli* can be monitored with the tracking microscope[5], which follows the point on the surface of the cell closest to the centre of the laboratory reference frame. If the cell rotates counterclockwise ($\Omega > 0$), the transducer traces out a counterclockwise path. If the cell is seen from the side and is tethered near one end, the x and y velocities of the tracker vary with time roughly as $\Omega\sin\Omega t$ and $-\Omega\cos\Omega t$ (Figs 1 and 2). The exact shape of the waveform depends on the size and the shape of the cell and on the position of the rotation axis.

The motion can be remarkably smooth. If the motor makes one revolution in n discrete steps, I conclude from data of the kind shown in Fig. 1 (for cells rotating as slowly as 4 r.p.s.) that $n \gtrsim 25$. This probably rules out the mechanism envisaged earlier[2], in which a cycle is executed when each of three (or more) cross bridges takes nine steps. This mechanism could explain the data only if the motions of the different cross bridges were highly synchronised. If the response time of the tracking and recording system is τ, then the root-mean-square deviation in Ω due to rotational diffusion should be of order $(2D/\tau)^{1/2}$. Under the conditions of the experiment ($\tau \simeq 10^{-2}$ s), the value of the deviation expected for a freely rotating object the size of *E. coli* is about 6 s^{-1}. The data are much smoother than this (Fig. 1), so the coupling between the flagellum and the body of the cell is not fluid.

Changes in the direction of rotation[1] occur smoothly, yet abruptly (Figs 1 and 2), and they occur at different points in the cycle (Fig. 2 and data obtained with the polyhook mutant, not shown). The data at hand are consistent with a model in which the reversals occur at random[9]. The angular velocities for clockwise and counterclockwise rotation are generally different (Figs 1 and 2), but this does not necessarily mean that the dirve is asymmetric. When the motor reverses, the torque applied to the hook and the filament changes

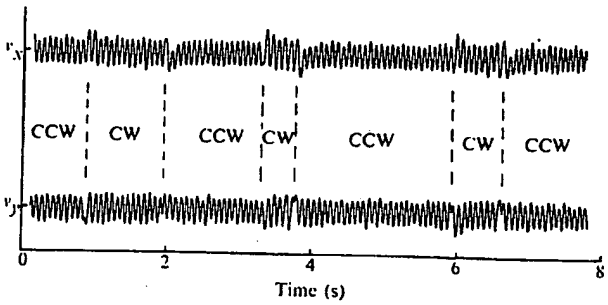

FIG. 2 The rotation of a wild type cell bound to the top window of the tracking chamber. In the interval shown the cell changed direction six times; it ran 71 cycles CCW at 13.2 ± 0.2 r.p.s. and 28 cycles CW at 12.4 ± 0.8 r.p.s. The reversals occurred at different points in the cycle. The cell was followed for a total of 6 min; the interval shown is typical of the entire record, except that the cell stopped occasionally for about 0.2 s and then started again in the same direction. *E. coli* strain AW405 (ref. 7) was grown, washed, and linked to the window by the methods of Larsen *et al.*[8] Other conditions were as described in Fig. 1.

sign; if either one changes its shape, the orientation of the cell (and thus the load) may be different. Note that the amplitudes of the signals (Fig. 1 or Fig. 2) are larger when the frquencies are lower.

Cells which rotate with larger radii of gyration tend to rotate more slowly, which implies that the motors slow down with increasing load. This was confirmed quantitatively in experiments with the polyhook mutant in which Ω was measured as a function of η (Table 1). When the motors run more slowly, they deliver somewhat more torque but less power. Since the torque is approximately constant, a cell tethered by an inert structure, such as a pilus (the point of attachment assumed fluid), and driven by a free helical filament (rotating rapidly on the opposite side of the cell) should rotate at about the same rate as a cell tethered by the flagellum itself (the point of attachment assumed rigid). I have seen this with wild type cells: in the absence of antibody, as many as 90% spin the wrong way (at the usual rates but predominantly clockwise on the slide, counterclockwise under the coverslip). As Silverman and Simon[1] note in the accompanying report, a distinction between rotation and other means of propulsion cannot be made with tethered cells unless they are non-motile when free.

Cells rotate perfectly well at high osmotic pressures, even when visibly plasmolysed (experiments with the polyhook mutant in 0.25 and 0.50 M sucrose). When the osmotic strength is increased, some cells slow down, but most continue to rotate rapidly (Ω in the range expected from changes in η). At higher osmotic strengths (0.75 M sucrose) most cells stop. These effects are reversible. The results are consistent with those obtained by Vaituzis and Doetsch[10,11], who found that flagellated spheroplasts swim in a medium isotonic to the cytoplasm provided that they retain remnants of the cell wall. Severe plasmolysis may cause reversible disruption of flagellar basal structures[12].

A model for a flagellar motor is presented in Fig. 3. The motor operates with two rings (the M and S rings), since only two have been found in Gram positive bacteria[13]. Four have been identified in Gram negative bacteria[13], but the outer pair probably serve as part of a bushing required for passage of the rod through the multilayered wall. The M ring has been shown to be associated with the cytoplasmic membrane in both Gram positive and Gram negative cells[14], but specific attachments of the S ring to known cell envelope structures have not been found. The motor must be attached to the wall somewhere, or else the torque which it can generate cannot be applied. If the rings are pulled away from the wall[12] or if the wall is dissolved away[11], the rings may continue to rotate relative to one another, but they will not drive the cell.

Lubrication does not seem to be a problem, the reason being that everything is very small. I have computed the torque due to the viscous shear in the space between the rod and the wall and in the space between the M and the S rings[3] and approximated the torque due to the rotation of the M ring in the membrane by half the torque on a sphere of similar radius. If the passage through the wall is 20 nm long, the gap between the M and the S rings is 0.5 nm thick, and the viscosity is 1 P (see Fig. 3 for the other dimensions), the internal drag is only 3×10^{-4} times as large as the drag on the body of the cell.

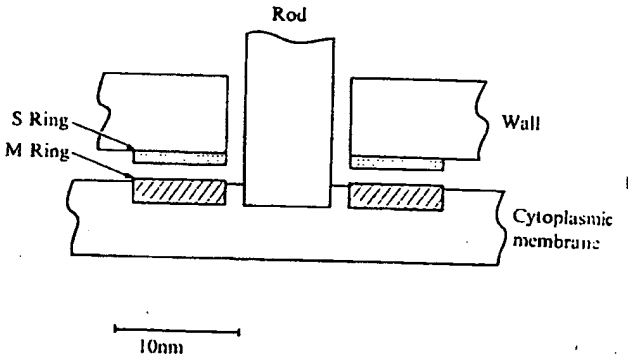

FIG. 3 A schematic representation of a flagellar motor. The rod is connected to a filament by a proximal hook (not shown). The torque is generated between the M ring and the S ring. The M ring, which is mounted rigidly on the rod (connections not shown), rotates freely in the cytoplasmic membrane. The S ring is mounted on the wall. The radius of the rod is 3.5 nm and the inner and outer radii of the rings are 5 nm and 11.2 nm, respectively[13]. (The wall thickness and the space between the M and S rings are not to scale.)

TABLE 1 Changes in angular velocity, torque and power dissipation resulting from changes in viscosity

Relative change in viscosity	2.42*	2.31†
Relative change in angular velocity	0.50 ± 0.08	0.51 ± 0.09
Relative change in external torque	1.21 ± 0.19	1.18 ± 0.21
Relative change in power dissipation	0.61 ± 0.22	0.60 ± 0.19

Cells were tethered to microscope coverslips by the method described in the legend of Fig. 1 and examined manually at 32°C. Cells rotating at uniform rates (0.3–3 r.p.s.) were clocked for about 1 min, the medium was replaced with one containing more (or less) methylcellulose (Dow Methocel 90 HG), and the measurements were repeated. Since the cells had different shapes and sizes and were tethered in different ways, the relative changes in Ω (and hence in the torque $b\eta\Omega$ and in the power dissipation $b\eta\Omega^2$) were computed for each cell separately. The data were obtained from twenty-one paired measurements on ten cells from four cultures. The standard deviations shown in the table were computed by weighting each pair of measurements equally. The viscosities were determined at 32°C with a Cannon-Ubbelohde viscometer.

* 0.776 to 1.88 cP.

† 1.88 to 4.35 cP.

The motor must bear both axial and radial loads, since the rod in a peritrichously flagellated bacterium rarely lies along the direction of motion. The forces are of order $6\pi\eta av$, where a is the radius of the cell (approximated as a sphere) and v is its speed; that is, of order 10^{-6} dynes. When an intact cell swims in a dilute solution, the axial load is more than balanced by osmotic pressure, which forces the M and S rings out against the wall (a force of order 10^{-5} dynes); when a spheroplast swims in a medium isotonic to its cytoplasm, the axial load is not balanced in this manner. As noted above, the coupling between the flagellum and the body of the cell

is not fluid, so the axial and the radial loads can be borne at the point of contact (between the M and the S rings).

How is the torque generated? Larsen *et al.*[15] have found that an intermediate in oxidative phosphorylation (but not ATP) is required for motility in *E. coli*. This suggests a mechanism in which the movement of one molecule down an electrochemical gradient (through the membrane) causes another molecule to exert a force on the S ring in a direction parallel to its face but normal to its radius. A force in the opposite direction could result from a change in the coupling between these molecules or from the action of an independent set. Since the power dissipation for an *E. coli* spinning 10 r.p.s. is of order 10^{-9} erg s^{-1} and the energy which can be gained from the transit of one molecule through the membrane is of order 10^{-13} erg, 10^{3} such events could drive the cell through one cycle. The individual steps would be too small to be seen in the present experiments.

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Presence of HCN in *Chlorella vulgaris* and its possible role in controlling the reduction of nitrate

NITRATE reductase of *Chlorella vulgaris* is present in crude extracts mainly in an inactive form[1–3] which may be converted to an active form by the addition of an artificial oxidant such as ferricyanide[4]. In crude extracts from which low-molecular weight substances have been removed by gel filtration, added NADH (or NADPH) causes a rapid conversion of the active form of nitrate reductase to the inactive form[5]. Losada and coworkers have also observed an inactivation of the nitrate reductase from *Chlorella fusca*[6] and *Chlamydomonas reinhardi*[7] by added NADH. A low-molecular weight fraction separated from crude extracts of *Chlorella vulgaris* also causes an inactivation of nitrate reductase when added back to the extract which had been treated with ferricyanide and passed through a Sephadex column to remove excess reagent[5]. In all cases the enzyme can be reactivated by the addition of the oxidant, ferricyanide[4–8]. On purification of the enzyme, Solomonson[9] found that two substances must be present simultaneously to cause the reversible inactivation: (1) a reducing substance, preferably NADH, and (2) a substance that behaves like HCN. The molar concentration of HCN needed to effect a rapid and reversible inactivation of the enzyme was of about the same magnitude as the enzyme concentration. Thus, activated, suitably purified nitrate reductase with added excess NADH, provides an exceedingly sensitive, though non-specific test for HCN, as shown in Fig. 1.

Although this bioassay could not be applied directly to extracts, it could easily be used to show that distillates prepared from *Chlorella* cells, or from extracts of *Chlorella* cells, contained something which behaved like HCN. The concentration of the unknown substance could be increased by redistillation until it came within the sensitivity range of specific chemical tests, which showed that the substance was, indeed, HCN. After many preliminary experiments, conditions were found for obtaining sufficiently high HCN concentrations in the first distillate to come within the range of a chemical test as well as of the bioassay. The results of a series of such experiments, given in Table 1, show that the amount of HCN which could be obtained from freshly collected *Chlorella* cells was somewhat greater than the amount of nitrate reductase present in the cells. Thus, the quantity of HCN found can account for the fact

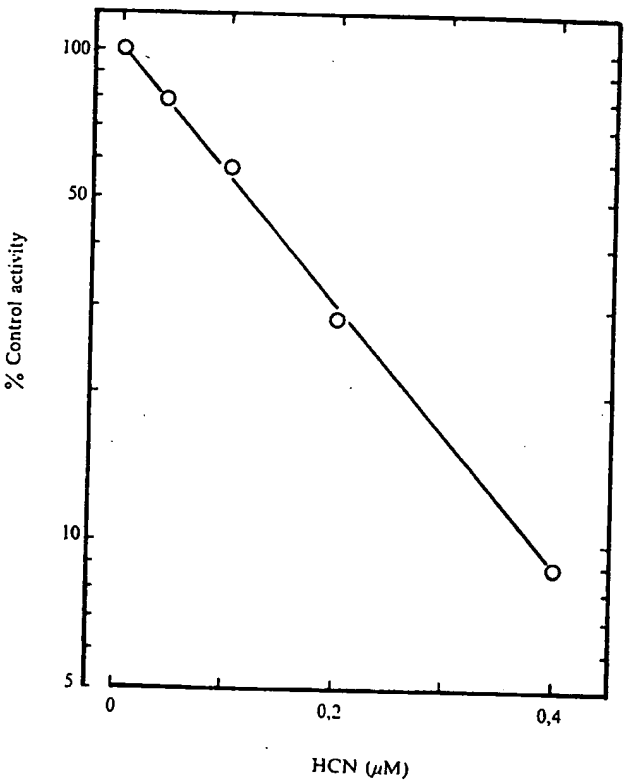

FIG. 1 A calibration curve for the bioassay for HCN. The percentage inactivation of nitrate reductase is plotted on a log scale against the initial HCN concentration. Incubation mixtures containing 0.05 M Na,K phosphate buffer (*p*H 7.6), 0.2 mM NADH, and the indicated concentrations of HCN were brought to 20° C. The inactivation was initiated by addition of activated enzyme to a concentration of 0.86 units ml^{-1}. After 6 min, aliquots were added to the enzyme assay mixture and activity was assayed as previously described[1,9]. A control inactivation mixture without cyanide, gave 95% of the initial activity. This was taken as 100% for calculation of the percentage inactivation. It is essential that all HCN be removed from the enzyme preparation, so that NADH alone gives a negligible inactivation. The preparation of the enzyme is described elsewhere[9]. The sensitivity of this bioassay can be increased by decreasing the concentration of enzyme.